ADRENALIN INDUCED TURNOVER OF PHOSPHATIDIC ACID AND PHOSPHATIDYL INOSITOL IN CHLORIDE CELLS FROM THE GILLS OF *ANGUILLA ANGUILLA*

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1. Introduction

α-Adrenergic stimuli cause an enhanced turnover of phosphatidic acid and phosphatidyl inositol in a variety of tissues from different animals [1]. Among the first tissues to be studied in this respect was the avian salt gland [2] a tissue that secretes a hypertonic saline. Functionally similar salt-secreting tissues are the rectal gland of elasmobranchs and the gills of marine teleosts. All these tissues contain chloride cells characterised by numerous mitochondria and an extensively infolded plasma membrane that appears as a smooth endoplasmic reticulum. The latter structure is highly enriched in $(Na^+ + K^+)$-dependent ATPase and has proved a very convenient starting material for isolation of the enzyme [3].

The present study was undertaken because our understanding of the physiology of salt-secreting epithelia, including the effects of adrenergic stimuli, is currently most advanced for the gills of marine teleosts [4,5]. Enriched populations of chloride cells can be isolated from eel gills [6] and their associated $(Na^+ + K^+)$-dependent ATPase has been partially purified and characterised [7].

Adrenalin (10^{-5} M) causes an enhanced influx of NaCl through the gills of freshwater teleosts through β-adrenergic stimulus [8] but causes a decreased outflux of NaCl through the gills of seawater teleosts through α-adrenergic stimulus [9]. The present report shows that adrenalin (10^{-5} M) does not stimulate the turnover of phosphatidic acid and phosphatidyl inositol in chloride cells from freshwater gills; it does, however, stimulate the turnover of these phospholipids in chloride cells from seawater gills.

2. Materials and methods

[^{32}P]Orthophosphate (36–83 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks, England.

The fish used were sexually immature eels (*Anguilla anguilla*) netted in the upper and lower reaches of the River Dee, Scotland. All the fish were acclimatized in either a freshwater aquarium or a seawater aquarium at 10–12°C for at least one month prior to use.

Labelling of phospholipids with [^{32}P]orthophosphate was performed in the isolated perfused gill preparation developed by Payan and Matty [10]. Briefly, all eight gill arches of a fish are perfused in situ via the heart with a physiological saline under constant pressure (50 cm H_2O). Either freshwater or seawater is gassed with air and pumped rapidly across the external gill surface at a rate of 1.2 l/min. Under these conditions NaCl is pumped across the gill epithelium at rates coinciding with those in vivo [8]. Satisfactory flow rates in the internal perfusion are obtained only in the presence of a 5×10^{-7} M adrenalin added to the internal saline. In the present work the phosphate buffer in the original saline of Payan and Matty [10] was replaced with HEPES and NaOH to maintain a high specific radioactivity in the added [^{32}P]orthophosphate. The modified medium

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used here contained, for freshwater eels: NaCl 114 mM, KCl 2 mM, $(NH_4)_2SO_4$ 0.4 mM, $NaHCO_3$ 20 mM, HEPES 2.5 mM, NaOH 3.0 mM, $CaCl_2$ 0.63 mM, $MgSO_4$ 0.2 mM, heparin 5000 IU/l, glucose 1 g/l, lactic acid 5 mM and polyvinylpyrrolidone 20 g/l and for seawater eels: NaCl 150 mM, K_2SO_4 0.2 mM, $(NH_4)_2SO_4$ 0.11 mM, $NaHCO_3$ 20 mM, HEPES 2.5 mM, NaOH 2.4 mM, $CaCl_2$ 0.63 mM, $MgSO_4$ 0.2 mM, urea 0.25 mM, heparin 5000 UI/l, glucose 1 g/l, lactic acid 5 mM and polyvinylpyrrolidone 20 g/l. The buffers were filtered through 0.22 μM millipore filters before use and were gassed with air 98%–CO_2 2% before and during the perfusion in order to maintain the pH at 7.65.

Initially, a perfusion of about 10 min without [^{32}P]orthophosphate saline was performed, in order to eliminate red blood cells. In all cases perfusion with [^{32}P]orthophosphate (2–3 μCi/ml) was carried out for 60 min at 15°C at a flow-rate of approximately 100 ml/h . 100 g fish. Saline without [^{32}P]orthophosphate (20 ml) was finally passed rapidly through the gills, which were then excised immediately into fresh, chilled saline.

Enriched population of respiratory epithelial cells and chloride cells were isolated from the gills by procedures involving mechanical separation of cells from the tissues and purification by density gradient centrifugation using Ficoll [6].

Total lipid was extracted from the two types of cells by the method of Folch et al. [11]. Individual phospholipid classes were separated by two-dimensional thin-layer chromatography on 250 μm layers of silic acid without binder (supplied by Analabs Inc., Subsidiary of New England Nuclear Corp., North Haven, Connecticut 06473, USA) using the solvent system of Parsons and Patton [12]. Total phospholipids were also analysed for mon-, di- and triphosphoinositides by the method of Gonzales-Sastre and Folch-Pi [13]. Phospholipids were detected by brief exposure of plates to iodine vapour after which individual zones were scraped from the plates into 10 ml of Instagel (Packard Instruments Inc., Caversham, Berks, England) and radioassayed in a Packard Series 3385 Tricard operated at 1.5% gain. Individual phospholipids were identified by reference to published R_F data [12] as well as by reference to authentic standards purchased from Sigma (London) Chemicals Co. Lts., Kingston-upon-Thames, Surrey, England.

The concentration of inorganic phosphate in gill tissue was measured by extracting known weights of the latter with 5% trichloroacetic acid and determining phosphate by the method of Martin and Doty [14]. Total protein was estimated by the method of Lowry [15], as modified by Schuel and Schuel [16]. Total phospholipid was determined by the method of Raheja et al. [17].

3. Results and discussion

Gills from seawater eels yielded populations of chloride cells containing twice as much protein and phospholipid as gills from freshwater eels; the respiratory cell population was unchanged (table 1). The data entirely support earlier conclusions that adaptation of freshwater eels to sea water is accompanied by the appearance of increased numbers of chloride cells in the gills leading to an enhanced activity of $(Na^+ + K^+)$-dependent ATPase [6,7,18,19]. That is, adaptation from fresh water to sea water causes an increased concentration of salt-pumping

Table 1
Level of protein and phospholipid in respiratory epithelial and chloride cell populations from the gills of freshwater and seawater eels

	Freshwater		Seawater	
	Protein	Phospholipid	Protein	Phospholipid
Respiratory epithelial cells	5118 ± 1142 (n = 5)	692 ± 107 (n = 8)	5004 ± 846 (n = 8)	683 ± 66 (n = 13)
Chloride cells	902 ± 172 (n = 5)	227 ± 51 (n = 8)	1987 ± 270 (n = 8)	509 ± 65 (n = 13)

Data quoted are μg/100 g fish ± SE. Values in parentheses are numbers of determinations.

Table 2
Percent distribution of radioactivity in phospholipids after perfusion of freshwater and seawater gills with [^{32}P]othophosphate in the presence of 5×10^{-7} M adrenalin

Phospholipid	Seawater (%)	Freshwater (%)
Phosphatidyl ethanolamine	6.8 ± 1.9 (n = 7)	3.2 ± 0.3 (n = 5)
Phosphatidyl choline	2.9 ± 0.8 (n = 7)	1.6 ± 0.5 (n = 5)
Phosphatidyl inositol	47.9 ± 2.6 (n = 7)	52.8 ± 3.1 (n = 5)
Phosphatidic acid	38.4 ± 3.8 (n = 7)	42.0 ± 3.1 (n = 5)
Sphyngomyelin	3.8 ± 3.1 (n = 7)	0.4 ± 0.1 (n = 5)

Specific radioactivities of total phospholipids (100% values) from seawater and freshwater perfusions were 9200 ± 700 (n = 3) and 17 400 ± 3700 (n = 3) cpm/mg phospholipid, respectively. These data are normalised to a 100 g fish perfused with 5×10^6 cpm of [^{32}P]orthophosphate/ml.

biomembranes in gills, in line with the fact that the salt-load pumped outwards across the gills in seawater is greater the salt-load pumped inwards in freshwater [4,5].

Table 2 documents the percentage distribution of radioactivity in gill phospholipids after perfusion of both freshwater and seawater preparations in the presence of [^{32}P]orthophosphate and 5×10^{-7} M adrenalin. Phosphatidic acid and phosphatidyl inositol account for nearly all the radioactivity incorporated and these lipids are labelled to essentially the same extent in both freshwater and seawater preparations. Separate analyses of gill phospholipids failed to reveal significant radioactivity in di- and triphosphoinositides.

The specific radioactivities of total gill phospholipids after perfusing freshwater and seawater preparations in the presence of 5×10^{-7} M adrenalin are shown in table 3. At this, the lowest concentration of adrenalin that can be used in the system, the specific activity of total phospholipids is twice as high in freshwater respiratory epithelial cells as in freshwater chloride cells. A similar situation holds for the seawater cell populations. Freshwater cell populations have essentially twice the specific radioactivity of the corresponding seawater preparations. The only significant change observed when perfusions are carried out in the presence of 10^{-5} M adrenalin is that the specific radioactivity of phospholipids in seawater chloride cells is essentially doubled to reach the value for freshwater chloride cells (table 3).

The percentage distribution of radioactivity in individual phospholipids in table 3 was the same as

Table 3
Specific radioactivities of total phospholipids in respiratory epithelial and chloride cell populations of gills of freshwater and seawater eels perfused with [^{32}P]orthophosphate in the presence of 5×10^{-7} and 10^{-5} M adrenalin

	Freshwater		Seawater	
	5×10^{-7} M	10^{-5} M	5×10^{-7} M	10^{-5} M
Respiratory epithelial cells	18 300 ± 1900 (n = 3)	17 500 ± 1700 (n = 3)	11 300 ± 2000 (n = 4)	14 800 ± 3700 (n = 5)
Chloride cells	9700 ± 1700 (n = 3)	10 600 ± 1000 (n = 3)	[a]5300 ± 2400 (n = 4)	[a]12 200 ± 2500 (n = 3)

[a] The values for seawater chloride cells are significantly different at $P < 0.01$. All other comparisons between 5×10^{-7} M and 10^{-5} M adrenalin are not significantly different.

Data are expressed as cpm ^{32}P/mg total phospholipid. The data were normalised to a 100 g fish perfused with 5×10^6 cpm [^{32}P]orthophosphate/per ml.

in table 2, irrespective of whether 5×10^{-7} M or 10^{-5} M adrenalin was used. Likewise the percentage composition of the gill phospholipids was constant throughout the experiments.

The concentration of inorganic phosphate in total gill tissue was 155 ± 9.6 ($n = 4$) μg phosphate/g wet wt gills for the freshwater animals and 134 ± 10.7 ($n = 5$) for the seawater animals. These values are not significantly different ($0.3 < p < 0.4$) and were the same whether adrenalin was present at 5×10^{-7} M or 10^{-5} M. Thus, the pool size of inorganic phosphate in the gills under the different experimental conditions remains constant. It is also known that the fluid space in gills is not increased at the higher adrenalin concentration; indeed the converse is true [20]. It follows that the increase in the specific activity of phospholipids in seawater chloride cells at the higher adrenalin concentration cannot be explained by an increased accessibility of chloride cells. Furthermore Girard [21] has shown that no change occurs in the pressure or the flow rate of perfusion when the concentration of adrenaline in the perfusion fluid is raised from 10^{-7} M to 10^{-5} M.

We conclude that the data here are entirely consistent with an enhanced rate of turnover of phosphatidic acid and phosphatidyl inositol promoted in the chloride cell fraction of seawater gills when perfused in the presence of 10^{-5} M adrenalin. Under these conditions, the effluxes of Na^+ and Cl^- are known to be inhibited by adrenalin. It has been shown on the seawater mullet that this inhibitory effect occurs through an activation of the α-adrenergic receptors [9]. The absence of an effect of adrenalin on the phospholipid turnover in the chloride cell fraction of the freshwater gills can be accounted for by the fact that, under these conditions, adrenalin promotes an increase of the sodium influx through the stimulation of a β-adrenergic receptor [8].

Whether increased turnover of phosphatidyl inositol alters the permeability of plasma membranes to monovalent cations, either specifically for chloride cells or generally for tissues susceptible to α-adrenergic stimuli, remains to be seen. Irrespective of the outcome the relatively simple physiological function of chloride cells and their susceptibility to α-adrenergic stimuli makes them a highly suitable material to probe the role of phosphatidyl inositol in biomembrane permeability.

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